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Analysis of Microbiological Screen Test Data for Antimicrobial Residues in Food Animals

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ABSTRACT

This study analyzes the National Residue Program (NRP) of the Food Safety and Inspection Service (FSIS), United States Department of Agriculture (USDA), data for the years 1983–1998 to determine the effectiveness of all three microbiological screen tests that were developed and used by the FSIS to control antimicrobial residues in food animals. The Swab Test On Premises (STOP) was the first screen test introduced in slaughterhouses, followed by the Calf Antibiotic Sulfonamide Test (CAST) and the Fast Antimicrobial Screen Test (FAST). The data for STOP indicates that during 1983–1998, the rate of food animal carcasses with violative levels of antimicrobial residues reduced from 2.33% to 0.45% under the monitoring plan and under the surveillance plan, the rate reduced from 55.1% to 0.56%. Similarly, the data for CAST indicates that the rate of calf carcasses with violative levels of antimicrobial residue also declined significantly during those years. Because of its higher sensitivity and shorter analytical time, the use of FAST started in 1995. By 1999, it had practically replaced the use of STOP and CAST in bovine species. The

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use of only one test such as FAST instead of different tests has removed confusion for testing different species of food animals and thereby has enhanced the efficiency of the NRP.

Key Words: Food animals; Residues; Sulfonamides; Antibiotics; Pilot study In-vitro; Screen tests; STOP; CAST; FAST.

INTRODUCTION

The use of antimicrobials such as antibiotics and sulfonamides as feed additives for growth promotion[1] and prevention of disease in food animals is a common practice.[2–4] At times, due to the nature of an antimicrobial, the species of animal exposed to the antimicrobial, and specifically for not adhering to the recommended ''withdrawal time'' following exposure to an antimicrobial, violative levels of antimicrobial residue may remain in the tissues of animal carcasses.[5] The consumption of such food animal tissues containing antibiotic and sulfonamide residues may adversely affect human health.[6] Thus, the National Residue Program (NRP) of the Food Safety and Inspection Service (FSIS), United States Department of Agriculture (USDA), designs a yearly plan to test the appropriate number of carcasses to prevent the entrance the violative levels of antibiotic and sulfonamide drug residues and agricultural chemicals into the human food chain through meat and poultry.[7,8] The NRP primarily conducts two types of residue testing programs in food animals at the point of slaughter. Under the monitoring program, a statistically significant number of samples from normal animal populations is analyzed on a national basis to detect a 1% residue violation at a 95% confidence level. Samples are taken from healthy animals that have passed inspection. The surveillance program, on the other hand, measures the magnitude of residue problems in a given population; including the effect of intervention measures by obtaining samples of individual animals or lots based on ante-mortem clinical signs, herd history or post mortem findings. Additionally, the NRP conducts exploratory residue testing and enforcement testing. The program annually publishes the *Domestic Residue Data Book* with results of analyses, which indicate the effectiveness of the program and the impact of changes in program operation and policy.

In support of the NRP, three microbiological screen tests were developed by FSIS scientists in order to detect antibiotics and sulfonamide residues in food animal carcasses. They include the Swab Test on Premises,[9,10] the Calf Antibiotic and Sulfonamide Test[11] and the Fast Antimicrobial Screen Test,[12] which have been used for monitoring and surveillance testing. The performance of all these screen tests has been reported,[13–15] their use reviewed[16,17] and their impact on the violation rate in food animal carcasses with agricultural residue has been reported.[18]

The Swab Test On Premises, more widely known as STOP, was developed in 1977 by FSIS laboratories to screen large numbers of meat samples.[10] In 1980, the test was modified for use in slaughter establishments by trained FSIS inspectors.[9] The procedure for the test conducted in a slaughter establishment is as follows: After a carcass has been identified, both kidneys from a carcass are collected. A sterile cotton tipped applicator is inserted into one of the kidney and left for 30 minutes to absorb the

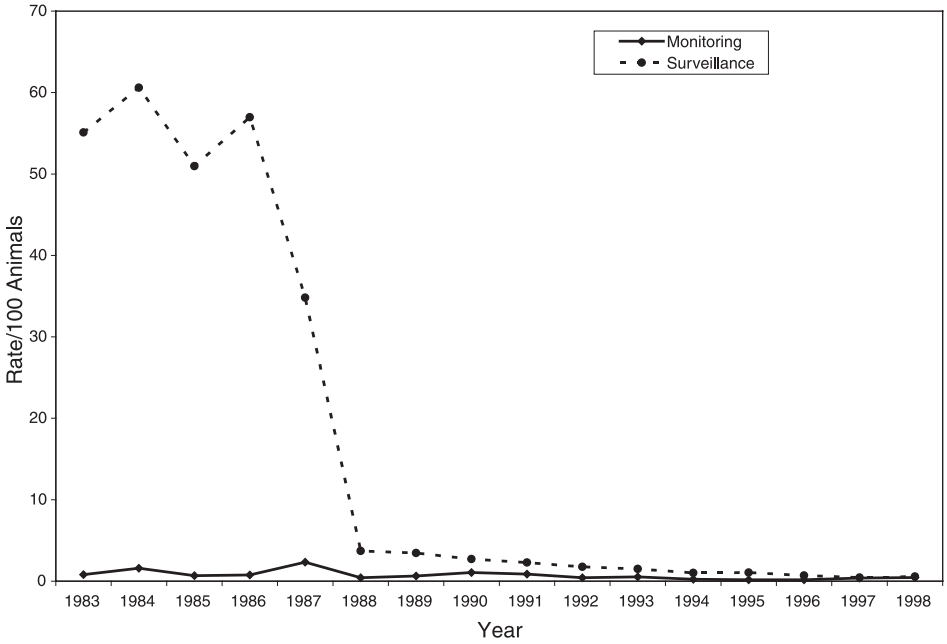

Figure 1. Rate of food animal carcass kidneys with violative level of antimicrobial residues by STOP screen test.

tissue fluid. A spore suspension of *Bacillus subtilis* is applied to the test plate with a fresh sterile swab (Figure 1). The swab from the kidney is removed, broken about 1/2 to 3/4 inches below the saturated cotton tip, and placed on the plate. Another swab from the kidney of another animal is placed on the same plate in a ''rabbit ear'' like formation. A standard neomycin 5 μg disc is placed on the lower third of the plate (Figure 2). After 16–24 hours of incubation at 37°C, the bacterial growth on the plates is examined. If the zones of inhibition are more than 1 mm wide from the edge of the swab to the edge of the zone is noted, presence of an antimicrobial is suspected in the carcass and the carcass is kept on ''hold.'' In such cases, samples of the kidney, liver, and muscles are sent to a FSIS laboratory for confirmatory analysis.[19] Depending on the analytical findings, the disposition of the carcass is made. Originally, STOP was used only on dairy cattle, but later it was used in all food animals and poultry, except ''bob'' veal calves.[11]

All three screen tests, namely STOP, CAST and FAST are modified microbial inhibition assays. For more than 15 years, depending upon the species and age of animal and the nature of antimicrobial use, one or the other screen has been applied to identify carcasses with violative levels of residual antimicrobials.[20] In all three tests, if the sampled kidney tissue fluid was found to inhibit the growth of the test organism on the plate and create a zone of inhibition around the swab, the muscle tissue of the carcass was suspected to contain violative level of antimicrobial residues. The muscle, liver and kidney tissues from the carcass were then subjected to confirmatory testing known as bioassay. Confirmatory bioassays performed on the tissues of suspected

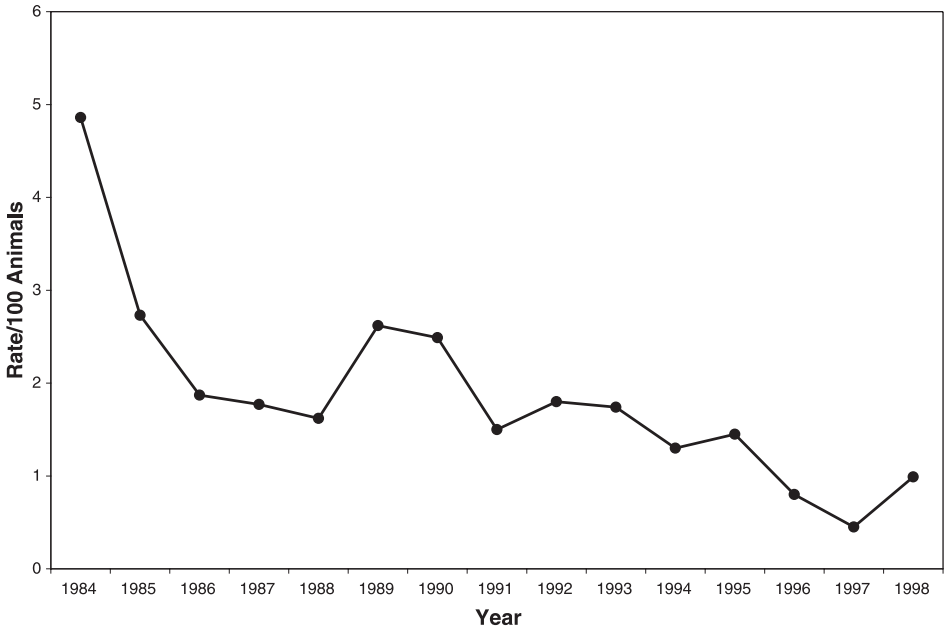

Figure 2. Rate of calf carcass kidneys with violative level of antimicrobial residues by CAST screen test.

carcasses over the past 15 years have established a positive correlation between the screen tests and the laboratory analysis.[a]

In the 1980's, an increase in the violation rate for sulfonamide and antibiotic residues in veal calf carcasses was noted. The incidence of violative levels of sulfonamide residue in ''bob'' veal carcasses (calves less than 3 weeks of age or 150 pounds in weight), rose sharply in 1981 and continued to increase in 1982.[a] The problem appeared to be widespread throughout the Northwest, Mid-Atlantic and upper Midwest. To ensure the safety of meat and other food products, in 1984, the Food and Drug Administration, under section 512 of the Federal Food, Drug and Cosmetic Act (21 U.S.C 360 b), set a tolerance level for sulfamethazine and sulfathiazole in tissues at 0.1 ppm (parts-per-million). Subsequently, the evidence of greenish discoloration of the stomachs of slaughtered calf carcasses, aptly called ''green gut'' carcass, alerted the USDA inspector to sample them for possible drug sulfonamides in ''bob'' veal calves. In order to screen such animals, the Calf Antibiotic and Sulfa Test (CAST) was developed, and in 1985 the test was introduced in slaughter establishments for the detection of antibiotic and sulfonamide residues in bob veal calf carcasses.[21] The procedure for performing CAST is identical to STOP except that the test organism is *Bacillus megatarium*, and the incubation temperature for the test plate is 41°C.[14]

[a]United States Department of Agriculture. Food Safety and Inspection Service. *Domestic Residue Data Book*. National Residue Program, 1981–1983. Washington, D.C.

Because neither STOP nor CAST was sensitive enough to detect sulfonamides at a violative level, the Fast Antimicrobial Test (FAST) was developed in 1994.[13] One pilot study of FAST in the bovine species,[22] and another in swine (not reported), show that carcasses can be screened by FAST in six hours.[12,15] It means that results with FAST can be visualized after 6 hours of incubation of the plate, and zone of inhibition remains unchanged till 18 hours of incubation. The procedure for performing FAST is similar to CAST except that the medium used in the FAST test plates is different from CAST, and the results with FAST can be observed sooner. Following the pilot study,[22] FSIS initiated the use of FAST to replace CAST in calf carcasses.[23] In addition to obtaining results in 6 hours, FAST is more sensitive to sulfonamides than CAST and can detect a greater variety of antibiotics and sulfonamides, than STOP (not reported earlier). Therefore, carcasses tested by FAST can be released earlier than those tested either by STOP or CAST. Following the successful testing of FAST in culled dairy cows in 1994, NRP data indicate that FAST was being used more and more to replace CAST and STOP.

This study analyzes the NPR data for STOP, CAST and FAST screen tests used at all federally inspected slaughter houses for the past 15 years for their association with reduced rates of antibiotic and sulfonamide residues that FSIS tests for in food animals. Additionally, the study attempt to determine the impact of using only one test by comparing the in vivo performance all three screen tests.

MATERIAL AND METHODS

NRP Domestic Residue Data Book[b]

The data published annually in the FSIS Domestic Residue Data Book on the antibiotic and sulfonamide residues detected in all bovine, swine, and ovine species and in poultry for the years 1983–1998 were used for this study. On the basis of the total number of animals and birds slaughtered, carcasses were sampled for antibiotic and sulfonamide residue analysis. From the number of samples found to contain a violative level of antimicrobial residue, a yearly incidence rate of violations was determined.[24]

Fast Field Study

The report entitled ''Fast Antimicrobial Screen Test (FAST) In-Plant Study:-A Report[22] by the United States Department of Agriculture, Food Safety and Inspection Service, Washington, D.C., describing the field study with FAST was also used as a supporting document for this study.

Sensitivity of Screen Tests

The antimicrobial sensitivities of the STOP, CAST, and FAST were evaluated by determining the minimum inhibitory concentration (MIC) of these tests to different

[b]United States Department of Agriculture. Food Safety and Inspection Service. *Domestic Residue Data Book*. National Residue Program, 1983–1999. Washington, D.C.

antibiotics and sulfonamides as determined in the FSIS Antibiotic Residue Laboratory, Beltsville, MD. This data was not reported earlier, however, it was analyzed here in order to support the findings of the field study data of the screen tests.

PROCEDURE

Analysis of the NRP Data

The residue data for STOP and CAST, published in the FSIS Domestic Residue Data Book of the NPR for the years 1983–1998 was analyzed for the incidence rate of antimicrobial residues in food animals (Tables 1 and 2).

Analysis of the Fast Field Study Data

The field study conducted in 1993 tested 479 calf kidneys with CAST, as well as FAST, and 292 kidneys with STOP, as well as, FAST. Muscle, liver, and kidney samples from carcasses whose kidney tissue fluid produced a zone of inhibition by screen tests, were confirmed by bioassay.[22] Extrapolated data for true and false positives and negatives, test sensitivity, and specificity were analyzed for this study (Table 3).

Table 1. Antimicrobial residue violations in food animal kidneys under monitoring and surveillance programs detected by STOP during 1983–1998.*

	Programs					
	Monitoring			Surveillance		
Year	Samples	Violations	Violation rate	Samples	Violations	Violation rate
1983	8721	70	0.8	1722	949	55.1
1984	4220	68	1.6	3148	1908	60.6
1985	5235	35	0.67	1636	834	50.97
1986	4616	36	0.77	3273	1865	56.98
1987	4326	101	2.33	5300	1846	34.83
1988	4849	21	0.43	56,546	2108	3.72
1989	8038	52	0.64	85,083	937	3.45
1990	7279	78	1.06	118,533	3200	2.71
1991	5909	51	0.86	117,850	2701	2.29
1992	4044	17	0.42	124,461	2206	1.77
1993	8274	42	0.52	121,043	1835	1.51
1994	8047	19	0.23	102,521	1046	1.02
1995	8687	15	0.17	83,524	888	1.06
1996	7373	13	0.18	41,995	292	0.7
1997	7977	30	0.38	33,709	148	0.44
1998	7829	37	0.47	37,633	220	0.58

*United States Department of Agriculture. Food Safety and Inspection Service. *Domestic Residue Data Book*. National Residue Program, 1984–1999. Washington, D.C.

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Table 2. Antimicrobial residue violations in calf kidneys during 1984–1998 detected by CAST.*

Year	Number of samples	Violations	Violation rate
1984	38,853	1891	4.86
1985	91,932	2510	2.73
1986	156,378	2930	1.87
1987	204,222	3615	1.77
1988	168,210	4599	1.62
1989	175,427	4599	2.62
1990	115,403	2070	2.49
1991	79,666	1196	1.5
1992	111,833	2021	1.8
1993	65,590	1084	1.74
1994	65,059	948	1.3
1995	58,197	848	1.45
1996	21,.045	169	0.8
1997	11,988	55	0.45
1998	8958	89	0.99

*United States Department of Agriculture Food Safety and Inspection Service. *Domestic Residue Data Book*. National Residue Program, 1984–99. Washington, D.C.

Determination of Sensitivity of Screen Tests

Five concentrations of an antimicrobial were prepared in Butterfield's phosphate buffer. An aliquot of 25 microliters of a solution was pipetted onto each 1/4 in analytical paper discs (Schleicher & Schuell # 740-E, Fisher,) so that a set of discs had the same desired concentrations of the antimicrobial agent. Similarly, other paper discs

Table 3. Comparative evaluation of STOP vs. FAST and CAST vs. FAST with 479 field data subjected to laboratory analysis.*

	Screen tests			
Samples	STOP Vs	FAST	CAST Vs	FAST
No. positive	177	156	156	152
No. negative	302	323	136	140
No. false negative[a] (%)	19(9.7)	40(20.4)	3(1.90)	7(4.4)
No. false positive[b] (%)	94(33.20)	72(25.4)	75(56.4)	64(48.1)
Test specificity[c] (%)	66.8	74.6	43.6	51.9
Test sensitivity[d] (%)	90.3	79.6	98.1	95.6

[a]Tissues containing antimicrobial residues that were not detected by the screen test;
[b]True negative samples which are detected as positive by the screen test;
[c]Percentage of tissues correctly identified as not containing any antimicrobial residues;
[d]Percentage of tissues correctly identified as containing antimicrobial residues.
*(From Ref. [22].)

with different concentrations of other antimicrobials were prepared and dried at 37°C for 30 min and stored at −15°C. The discs were evaluated against commercially available antimicrobial discs. STOP, CAST, and FAST plates were prepared according to the described methodology.[13–15] Discs impregnated with various kinds and concentrations of antimicrobial agents were placed on STOP, CAST, and FAST plates in triplicate. Different plates were incubated at different temperatures.[13–15] After incubation, the zone of inhibition around each disc was measured.

RESULTS

The rate of residue violations for monitoring samples in slaughtered food animals, based on STOP screen test findings, from 1983 through 1998, was below 2 % except in 1987, when the rate was 2.33% (Table 1). Compared to the first five years, the incidence rate in monitoring samples for the last eleven years decreased (weighted mean 1.14 vs. 0.53).

During those years (1983–1998); the highest violation rate of 60.6 % from surveillance was noted in 1984 (Table 1). The rate based on 37,633 samples decreased to 0.58% in 1998 ($P < 0.001$). The trend indicates that the incidence rate with surveillance samples since 1991, compared to the incidence rate before 1991, decreased significantly (average mean 2.20 vs. 1.6).

The residue violation rate per 100 calf carcasses determined by CAST was 4.86 in 1985, which decreased steadily to 0.99 by 1998, except in 1989 when it increased to

Table 4. Sensitivity of STOP, CAST and FAST screen tests to various concentrations of antimicrobials.

	Screen tests		
Antimicrobial	STOP[a]	CAST[a]	FAST[b]
	Sensitivity to the concentration (μg)		
Neomycin	0.025	0.0125	0.0125
Gentamicin	0.125	0.0063	0.0063
Penicillin G	0.0125	0.0125	0.0125
Streptomycin	0.125	0.125	0.125
Tetracycline	0.125	0.125	0.125
Chlortetracycline	0.0315	0.0315	0.0315
Oxytetracycline	0.125	0.125	0.125
Erythromycin	0.0315	0.0315	0.0315
Tylosin	0.125	0.125	0.125
Sulfamethazine	NI[c]	2.0	0.5
Sulfathiazole	NI	1.0	0.5
Sulfadimethoxine	NI	0.25	0.125

[a]Lower limit of detection (LLD) values determined after 18 hours of plate incubation:
[b]Lower limit of detection (LLD) values determined after 6 hours of plate incubation;
[c]No inhibition at all concentrations tested up to 2.0 μg/disc.

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2.67 (Table 2). The rate of violative carcasses indicates that a gradual decrease since the screen test was first used in 1985.

To have a comparative perspective of the significant decreases in the rate of violation for the years 1983–1998 in both surveillance and monitoring samples for all food animals and in calves (Tables 1 and 2), the data is presented graphically (Figures 1 and 2). Although significant, the change in violation rate in monitoring samples was not as dramatic as the reduction in the violation rate in surveillance samples (Figure 1). The CAST data indicate that the violation rate in calf carcasses for the years 1989 and 1990 was high compared to subsequent years (Figure 2).

The pilot study data comparing the efficacy of all three screen tests; that is STOP, CAST and FAST, show that the antimicrobial residue detection rate by FAST is significantly different from STOP, but not from CAST (Table 3). The overall field test results for the positive tissues detected by both FAST and CAST, show that the FAST performance is not significantly different than CAST ($p = 0.22$).

A laboratory analysis of all three screen tests for their sensitivity against all antimicrobials (Table 4) shows that both FAST and CAST have similar ranges of sensitivity against all the antibiotics tested. The Least Level of Detection (LLD) by FAST for antibiotics was similar to the LLD by CAST but for sulfonamides was better than CAST. This indicates that FAST is superior to CAST for detecting sulfonamide residues, even though CAST was specially developed for use in bob veal carcasses. Additionally, the LLD values of FAST for antibiotics and sulfonamides as a group, compared with STOP, clearly show that FAST is also a better screen test for residue detection in adult bovine species samples.

DISCUSSION

In the 1970's, the USDA began testing for residual antibiotics in meat and poultry[10] by slightly modifying a FDA procedure used for testing antibiotic residues in milk.[25] The samples collected at the slaughter establishments were analyzed in a FSIS laboratory for violative levels of antibiotic residues. While the sample was being analyzed, the carcass was kept on ''hold'' by the USDA inspector. It usually took an average of 5 to 6 days for results. The procedure had lengthy carcass disposition time that increased with the complexity of the analysis. To minimize the time required for final carcass disposition, the STOP test was modified so that the test could be performed in slaughter establishments by a trained inspector. This allowed release of a majority of sampled carcasses because most carcasses that did not contain violative levels of antimicrobial residues.

In 1980, it was noted that the effort to use the STOP screen test for the detection and control of sulfonamide residues most prevalent in bob veal calves was not successful, because the sensitivity of STOP test to sulfonamides was minimal. As a result, the CAST screen test was introduced in 1984 especially to test bob veal carcasses. Over the years, the use of CAST supported condemnation of large numbers of carcasses. As a result, farmers became judicious in the use of sulfa drugs in calves and the sulfonamide residue violation rate in bob veal carcasses reduced dramatically (Figure 2). However, the test was not sensitive enough to detect sulfonamide residues at violative levels, which are very low levels. Thus, to identify bob veal calves rapidly

so that carcass disposition time can be reduced, the FAST screen test was introduced in 1995. Sensitivity to antimicrobials in tissues and to disks impregnated with pure chemical show that FAST has the ability to detect a wide range of antimicrobials, has higher sensitivity for most antimicrobials used in agriculture compared to STOP, and better sensitivity than CAST for sulfonamides (Table 4).

The STOP, CAST or FAST was used to screen a group of animal carcasses for violative levels of antimicrobial residues by testing a few of the animals from that group. A violative level is defined as the concentration that exceeds the FDA tolerance limit for an antimicrobial drug in a species, and in a particular tissue. The STOP monitoring data indicate that the violation rate in animals other than bob veal calves in the past 16 years did not change appreciably except in 1987 when the rate was 2.33% (Table 1). However, during this period the violation rate in surveillance samples decreased significantly (55.1% vs. 0.58%). Similarly, the data from CAST testing indicate that the rate of residue violation in calves decreased significantly (4.86% vs. 0.99%). This gradual decline in the number of violative calf carcasses tested with CAST indicates that the rate of sulfonamide residues also declined.[24] The gradual decline in the violation rate in both monitoring and surveillance samples tested with CAST, were also reported earlier.[26] The rise in the 1989 rate in monitoring samples (Figure 1) and in bob veal calves CAST (Figure 2) may have resulted from the use of newly introduced antimicrobial medication that did not clear the tissue by the time the animal was slaughtered.

The increase in the use of FAST to replace STOP and CAST following the pilot study as a better screen test[21] is supported by the in vitro study results. The data clearly show that FAST has higher sensitivity and better capability to detect wider ranges of antimicrobials than STOP (Tables 3 and 4), and is superior in detecting lower concentrations of sulfonamide than CAST (Table 4). As the zone of inhibition with FAST remains unchanged till 18 hours of incubation, a carcass with no violative antimicrobial residues can be released within 8 hours of a normal working day or FAST can be used for sampling carcasses at the end of a working day that can be checked early the next day.

With the promising test results by FAST[20] the use of FAST increased while the use of STOP and CAST has decreased (Figure 3). Because of better sensitivity and shorter analytical time, FAST in conjunction with STOP was successfully used for testing 99,015 culled dairy cattle in 1994 and 1995. The trend in the rate of violations in cattle screened with FAST during 1994–1998 (Figure 4) was similar to the violations detected by CAST, but was higher than the detection rate found in those animals tested STOP. This indicates that FAST is detecting residue in calves at the same level as CAST and, performing better than STOP in detecting in adult cattle. Analysis of the historical data indicates that both STOP and FAST screen tests provided the impetus for increased producer awareness regarding proper use of medicated feed and especially for adherence to withdrawal time for drugs or medicated feed prior to slaughter. As a result, the residue violation rate in food animals has reduced drastically. The NRP data indicate that a majority of food animals passed for human consumption are free of antimicrobial residues. Only a small number of food animals may contain violative levels of antibiotic residue that have the potential to cause human health problems. All three screen tests have been valuable aids to the FSIS mission of protecting the public from undesirable residues. When viewed in its

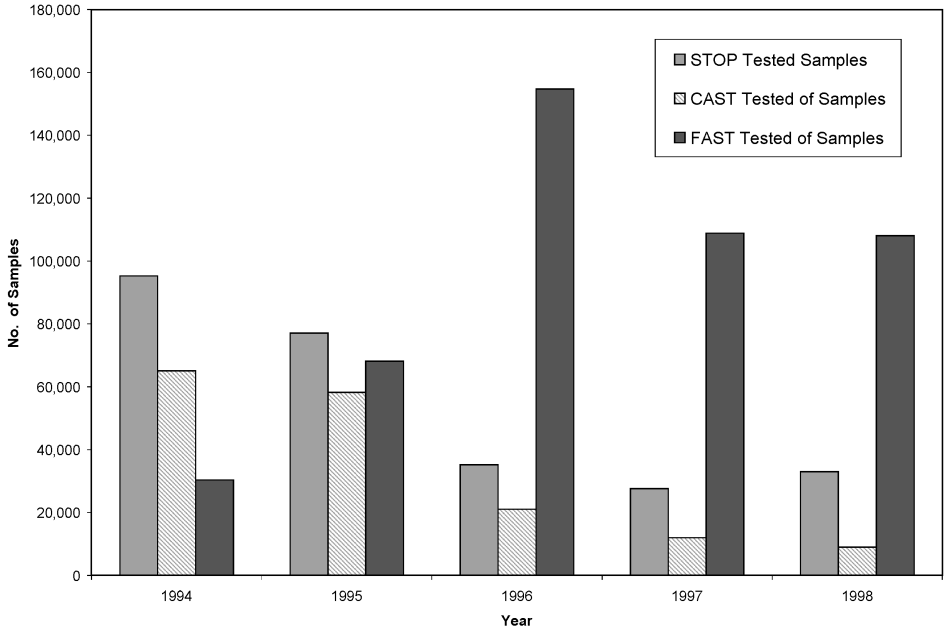

Figure 3. Number of STOP, CAST and FAST tests performed in bovine species during 1994–98.

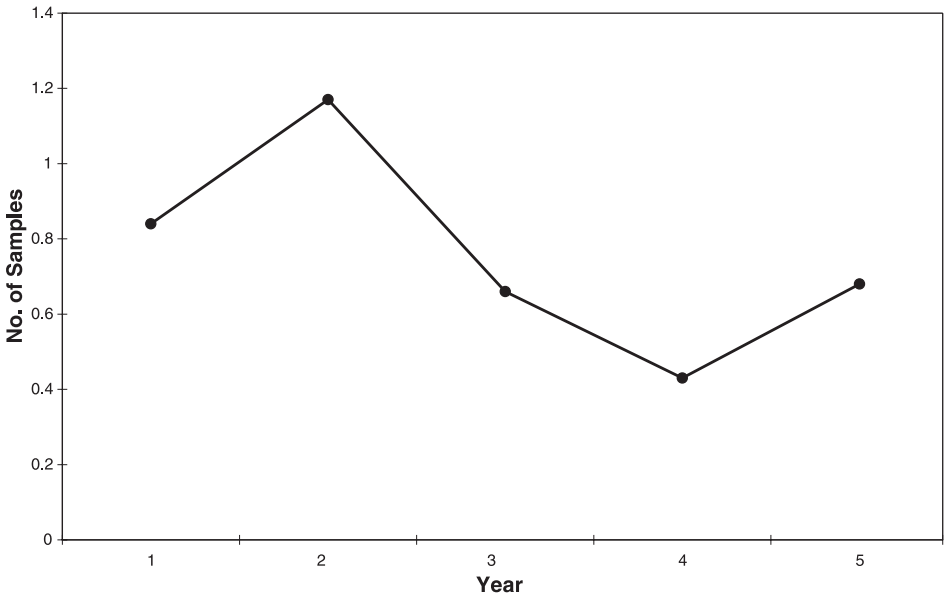

Figure 4. Rate of bovine carcass kidneys with violations level of antimicrobial residues by FAST during 1994–1998.

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totality, the use of STOP and CAST screen tests has been timely and useful. Since the first screen test was introduced in slaughtering plants in early 1980, it can be assumed that time for preparing and cost of mailing samples for laboratory confirmation has reduced. Above all, in the farm to table continuum, an antimicrobial screen tests such as FAST is an important tools to ensure that the federally inspected animal products are safe and free of harmful antimicrobial residues.

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